# Meiosis I kinase regulators

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# Meiosis I Kinase Regulators: Conserved Orchestrators of Reductional Chromosome Segregation

*Stefan Galander and Adèle L. Marston**

**Research over the last two decades has identified a group of meiosis-specific proteins, consisting of budding yeast Spo13, fission yeast Moa1, mouse MEIKIN, and *Drosophila* Mtrm, with essential functions in meiotic chromosome segregation. These proteins, which we call meiosis I kinase regulators (MOKIRs), mediate two major adaptations to the meiotic cell cycle to allow the generation of haploid gametes from diploid mother cells. Firstly, they promote the segregation of homologous chromosomes in meiosis I (reductional division) by ensuring that sister kinetochores face towards the same pole (mono-orientation). Secondly, they safeguard the timely separation of sister chromatids in meiosis II (equational division) by counteracting the premature removal of pericentromeric cohesin, and thus prevent the formation of aneuploid gametes. Although MOKIRs bear no obvious sequence similarity, they appear to play functionally conserved roles in regulating meiotic kinases. Here, the known functions of MOKIRs are reviewed and their possible mechanisms of action are discussed.**

## 1. Introduction

Eukaryotic cells proliferate and divide by one of two different modes. The mitotic cell cycle, which is used to generate the large majority of cell types, involves the separation of a single mother cell into two daughter cells that contain the same chromosomal content as the mothers. To achieve this, mitotic cells first replicate their DNA before segregating sister chromatids to opposite poles. During gametogenesis (see Appendix), however, cells undergo two successive cell divisions without an intervening round of DNA replication, leading to the halving of the chromosomal number. This process is called meiosis.

Due to the fundamentally different outcomes of mitotic and meiotic cell division, the chromosome segregation machinery has to be adapted in a multitude of ways to facilitate the desired outcome of the cellular division. In the vast majority of organisms studied, meiosis I is considered a specialized division because homologous chromosomes segregate (reductional division). Conversely, mitosis and meiosis II are equational divisions, meaning that sister chromatids are separated to opposite poles. A notable exception to this rule is plants with holocentric chromosomes (see Appendix), in which the typical sequence of reductional and equational division is inverted.[1]

The mitotic and meiotic cell cycles and chromosome segregation machineries differ in five key aspects (**Figure 1**), the molecular basis of which is largely still elusive.[2] First, in prophase I, homologous recombination creates linkages between homologous chromosomes and these connections are the basis for successful homolog segregation in meiosis I. Meiotic recombination is a complex process that involves the creation of doubl-strand breaks on chromosomes, the physical linking of homologous chromosomes within a structure called the synaptonemal complex (see Appendix), and the formation and resolution of Holliday junctions (see Appendix). Second, the events of meiotic recombination require that meiotic prophase be extended compared to mitotic prophase.[3] This prevents the premature expression of proteins that promote chromosome segregation and thereby ensures that segregation is only initiated once recombination is completed. Third, to segregate sister chromatids in mitosis and meiosis II, sister kinetochores (see Appendix), the proteinaceous structures mediating attachment of chromosomes to microtubules, need to face opposite poles (bi-orientation). In contrast, sister kinetochores must face toward the same pole (mono-orientation) to co-segregate in meiosis I. Fourthly, the cleavage dynamics of cohesin (see Appendix), the protein complex holding sister chromatids together, are altered in meiosis. Cohesin is cleaved on chromosome arms to allow the resolution of chiasmata (see Appendix) during anaphase I, which triggers the segregation of homologous chromosomes. However, cohesin in the regions surrounding centromeres (called pericentromeres) is retained until meiosis II to ensure the faithful segregation of sister chromatids in anaphase II. Finally, the meiotic cell cycle requires the fine-tuning of the activity of cell cycle kinases. Whereas mitotic cells eliminate the activity of cyclin-dependent kinases (CDKs) in anaphase I to promote exit from mitosis and allow the re-licensing of DNA replication origins (see Appendix), meiotic cells need to decrease CDK activity enough to drive exit from meiosis I while maintaining some CDK activity to suppress DNA

Dr. S. Galander[+], Prof. A. L. Marston
The Wellcome Centre for Cell Biology, Institute of Cell Biology
School of Biological Sciences
Michael Swann Building, Max Born Crescent, Edinburgh EH9 3BF, UK
E-mail: adele.marston@ed.ac.uk

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[+]Present address: The Company of Biologists, Bidder Building, Station Road, Histon, Cambridge, CB24 9LF, UK



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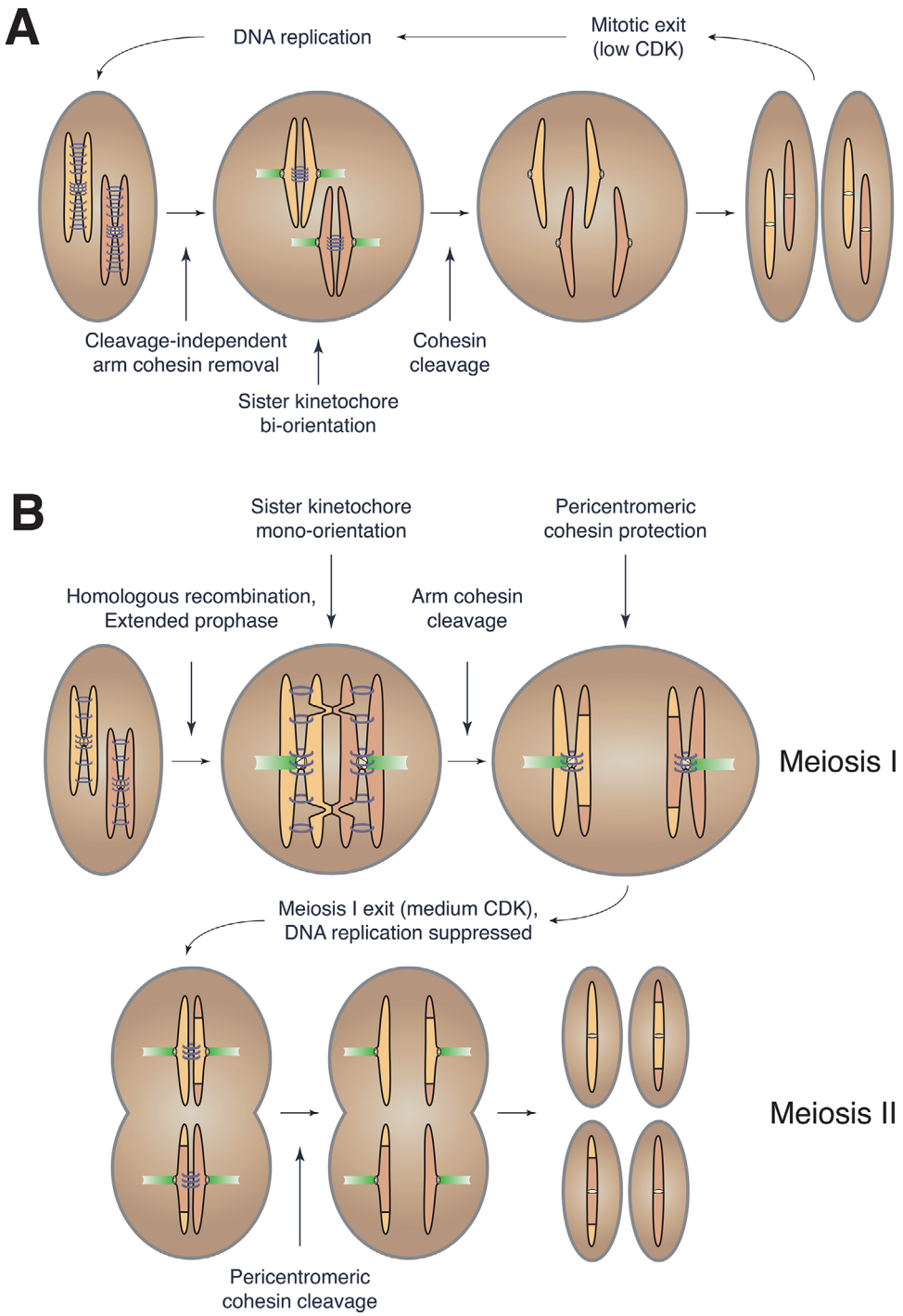



**Figure 1.** Comparison of mitotic and meiotic cell cycles. Mitosis and meiosis need to be specifically adapted to generate two diploid daughter cells or four haploid daughter cells from a single diploid mother cell, respectively. In the mitotic cell cycle (A), replicated sister chromatids attach to opposite spindle poles (bi-orientation). In mammals and *Drosophila*, cohesin, the protein complex that holds sister chromatids together, is removed from chromosome arms in prophase but retained in the pericentromere, where it resists the microtubule pulling forces to prevent sister chromatid segregation. In anaphase, cohesin is cleaved and sister chromatids move to opposite poles. This is followed by exit from mitosis and re-start of the cell cycle. In meiosis (B), prophase is extended to allow sufficient time for homologous recombination to occur. Recombination generates chiasmata, which link homologous chromosomes. Sister kinetochores are mono-oriented, meaning they face the same pole. When homologous chromosomes become attached by microtubules, chiasmata resist spindle forces and ensure the faithful segregation of homologs. Upon anaphase I onset, cohesin on chromosome arms is cleaved to allow the resolution of chiasmata and homolog segregation, whereas pericentromeric cohesin is retained. In the transition from meiosis I to meiosis II, DNA replication is suppressed. In meiosis II, pericentromeric cohesin is cleaved upon sister chromatid bi-orientation, and the meiotic exit programme is consequently initiated.

**Table 1.** Overview of key meiotic proteins involved in cohesin protection and kinetochore mono-orientation in various model organisms.

| Protein | Budding yeast *Saccharomyces cerevisiae* | Fission yeast *Schizosaccharomyces pombe* | *Drosophila melanogaster* | Mouse |
|---|---|---|---|---|
| Casein kinase 1 | Hrr25 (equivalent to CK1$\delta$ isoform) | Cki1 | Doubletime Dbt (CK1$\epsilon$ homolog) | CSNK1 |
| Dbf4-dependent kinase | Cdc7 (with regulatory subunit Dbf4) | Hsk1 (with Dfp1) | Cdc7 (with Chiffon) | CDC7 (with DBF4) |
| Polo kinase | Cdc5 | Plo1 | Polo | PLK1 |
| PP2A B' subunit | Rts1 | Par1 | Wdb and Wrd | B56 |
| Shugoshin (meiotic cohesin protector) | Sgo1 | Sgo1 | MEI-S332 | Sgo2 |

replication while promoting an additional round of chromosome segregation.

Both the mitotic and the meiotic cell cycle are driven by fluctuations in the activity of a number of kinases. The transition through interphase and cell division is mainly facilitated by CDKs, which associate with their cyclin co-factors to achieve temporal and spatial specificity for distinct cellular targets. In addition, mitosis and meiosis utilize a select number of kinases to promote cell cycle progression and accurate chromosome segregation (Table 1).[4] These kinases have multifaceted functions and direct chromosome segregation in a multitude of ways, implying that their activity needs to be strictly regulated. Evidence from a range of model organisms indicates that a group of meiosis-specific proteins that are seemingly unrelated in protein sequence control meiotic kinases to execute key features of the meiotic programme. These proteins include Spo13 (budding yeast), Moa1 (fission yeast), Mtrm (Matrimony; *Drosophila melanogaster*), and MEIKIN (mouse). Here, we review the known functions of these proteins, which we collectively refer to as Meiosis One Kinase Regulators (MOKIRs), and discuss the possibility that they are functionally conserved regulators that orchestrate the action of kinases in meiosis to ensure the accurate segregation of chromosomes.

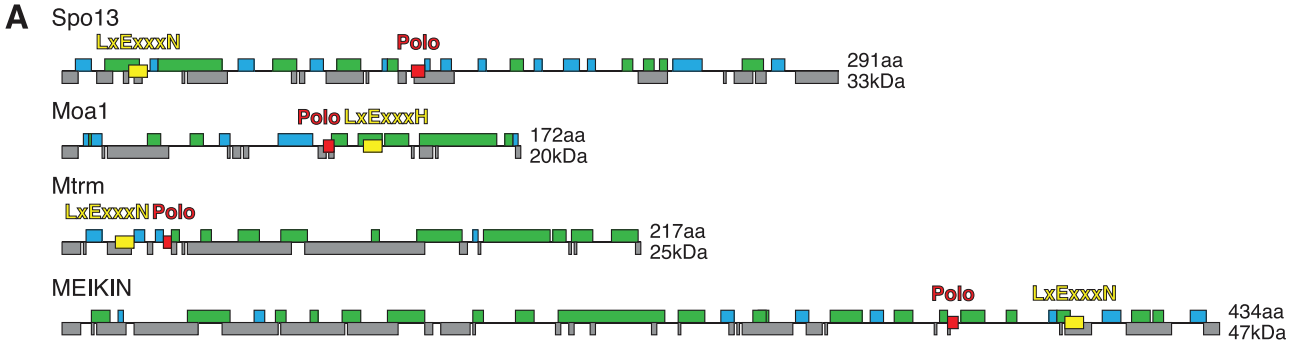
## 2. MOKIRs Show Poor Sequence Conservation

In terms of primary protein sequence, the conservation between MOKIRs is extremely poor. Accordingly, sequence alignment of the four proteins with Clustal Omega[5] does not identify any conserved regions or domains (data not shown). Nonetheless, these proteins share a number of common features (**Figure 2**A–C). Generally, they are small in size, ranging from 172 amino acids/20 kDa for Moa1$^{MOKIR}$ to 434 amino acids/47 kDa for MEIKIN$^{MOKIR}$. Secondary structure analysis with Phyre2[6] shows that they are characterized by large stretches of disordered regions (Figure 2A). The only notable exception is a sterile alpha motif (SAM)-domain found in the C-terminus of Mtrm$^{MOKIR}$, which is required to stabilize its binding to Polo[7,8] (see below). We speculate that a possible explanation for the lack of a clear 3D structure is that the shape of these proteins is largely determined by protein interactions, which could be facilitated by post-translational modifications, as is frequently observed for intrinsically disordered proteins.[9] Indeed, all four proteins carry a large number of serine and threonine residues (Figure 2B), which are targets for phosphorylation. Consistently, both Moa1$^{MOKIR}$[10] and Spo13$^{MOKIR}$[11,12] have been found to be phosphorylated.

The most notable example of the phosphorylation-induced interaction of MOKIRs with their partners is the presence of a Polo-box domain (PBD) binding region (Figure 2C) that requires prior phosphorylation, typically by CDKs, to interact with Polo kinase.[13,14] Indeed, Polo binding through the PBD-binding domain of MOKIRs has been demonstrated in mouse, *Drosophila*, fission and budding yeasts, and abrogation of the Polo-MOKIR interaction is detrimental for chromosome segregation in all cases.[11,12,15,16] In budding and fission yeasts, and mouse, MOKIRs recruit Polo kinase to kinetochores through the PBD-binding region, whereas in *Drosophila*, this region is important for the sequestration of Polo on the spindle (Figure 2D).[11,12,15,16]

Additionally, MOKIRs share a similar degradation motif (Figure 2C). This LxExxxN (short: LEN) motif has been shown to be required for the degradation of both Spo13$^{MOKIR}$[17] and Mtrm$^{MOKIR}$[18] by the anaphase promoting complex/cyclosome (APC/C) and ensures the degradation of these proteins in anaphase I[17] and at the oocyte-to-embryo transition,[18] respectively. Although failure to degrade Spo13$^{MOKIR}$ in a timely manner does not appear to affect the outcome of meiosis,[17] excess Mtrm$^{MOKIR}$ causes developmental defects in embryos.[18] MEIKIN$^{MOKIR}$ also possesses an LEN motif, whereas Moa1$^{MOKIR}$ carries an LxExxxH sequence; however, whether these motifs direct APC/C-mediated degradation of MEIKIN$^{MOKIR}$ and Moa1$^{MOKIR}$ remains to be determined.

A recent analysis of Mtrm$^{MOKIR}$ orthologs in *Drosophila* species has provided evidence that MOKIR functionality relies on only short segments of sequence conservation. Mtrm$^{MOKIR}$ protein sequences are highly diverse: in the most extreme example, Mtrm$^{MOKIR}$ of *D. melanogaster* and *D. grimshawi* only share 38.2% overall sequence identity.[8] Even the SAM-domain, which stabilizes the interaction of Mtrm$^{MOKIR}$ with Polo,[7] and the SAM proximal region, show only intermediate levels of conservation between these species (46.9% and 57.7%, respectively). Apart from these two regions, and the short PBD-binding region, sequence conservation is very poor, suggesting rapid evolutionary divergence between Mtrm$^{MOKIR}$ proteins in *Drosophila*. Despite this, expression of *D. grimshawi* Mtrm$^{MOKIR}$ rescues the meiotic defects in *D. melanogaster* carrying a heterozygous *mtrm* mutation (*mtrm* is haploinsufficient (see Appendix)).[8] Similarly, artificial targeting of Spo13$^{MOKIR}$ to fission yeast kinetochores rescues the mono-orientation defect observed in *moa1*Δ cells.[15] Thus, despite their strong evolutionary divergence at the sequence level, it is possible that MOKIRs have retained similar conserved molecular functions.




**B**

| | α-helix | β-sheet | Disorder | S/T |
|---|---|---|---|---|
| Spo13 | 29% | 20% | 37% | 16% |
| Moa1 | 39% | 15% | 29% | 17% |
| Mtrm | 43% | 7% | 53% | 17% |
| MEIKIN | 40% | 9% | 45% | 20% |

**C**

| | Polo | LxExxxN |
|---|---|---|
| Spo13 | EQPPKST**STP**VLSQS | LQEKTPN |
| Moa1 | KLKPVAT**STP**KSCMR | LNETVKH |
| Mrtm | NTSNVRC**STP**IFGNF | LKERRWN |
| Meikin | QSKALLT**STP**SSDIA | LQESSSN |

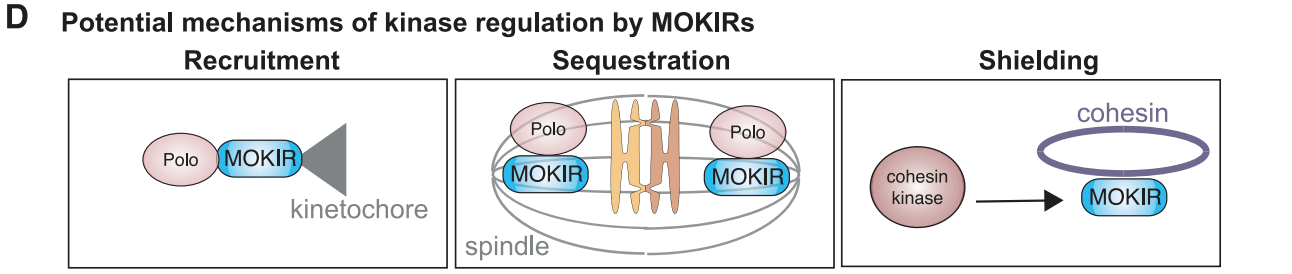



**Figure 2.** Structural comparison of MOKIRs and potential mechanisms of kinase regulation. MOKIRs are largely disordered proteins that show very little sequence conservation amongst each other. A) We used Phyre2 to model their secondary structure. Green and blue bars represent predicted $\alpha$-helical domains and $\beta$-sheets, respectively. Grey bars indicate disordered regions. The putative Polo-binding site in each protein is highlighted in red and the putative LEN motif for degradation by the APC/C is highlighted in yellow. B) Table comparing the properties of MOKIRs, as suggested by modelling with Phyre2. Of note is the larger than average content of serine and threonine residues, allowing for large-scale post-translational phosphorylation of these proteins. C) Alignment of Polo-binding sites and LEN motifs in MOKIRs. D) MOKIRs regulate meiotic kinases and several mechanisms have been proposed. In mouse, budding and fission yeasts, MOKIRs recruit Polo kinase to kinetochores, where they have roles in mono-orientation and, in some cases, cohesin protection. In *Drosophila*, Mtrm$^{MOKIR}$ appears to inhibit active Polo kinase by sequestration to the spindle. In budding yeast, Spo13$^{MOKIR}$ appears to prevent ectopic kinase activity from enhancing cohesin cleavage, and localizes to chromosomes in a cohesin-dependent manner. We speculate that Spo13$^{MOKIR}$ may shield cohesin from its kinases, though there is no direct evidence for this. Note that MOKIR interaction with kinases may be inhibitory or activating at specific locations and the three mechanisms above are not mutually exclusive for individual MOKIRs.

## 3. MOKIRs Regulate Cohesin Kinases and Shugoshin to Promote Cohesin Protection

### 3.1. Cohesin Protection Requires that Cohesin-Directed Kinase and Phosphatase Activity be Balanced

All MOKIRs appear to ensure the step-wise loss of cohesin, a defining feature of the meiotic chromosome segregation pattern (**Figure 3**). Cohesin is a ring-shaped complex that topologically links the two newly-duplicated sister chromatids as they are replicated to provide the cohesion that holds them together until the time of their segregation. Cohesin additionally links distant loci of the DNA molecule to structurally organize the chromosome, which affects multiple processes, including meiotic recombination.[19,20] Although a non-proteolytic cohesin removal pathway requiring the Wapl protein has been identified,[21–24] the universal trigger for chromosome segregation in both mitosis and meiosis is the proteolytic cleavage of the kleisin subunit of cohesin by separase.[19] During meiosis in the majority of organisms, sister chromatids are held together by cohesin complexes containing the meiosis-specific kleisin Rec8, which must be cleaved to trigger chromosome segregation.[25] Rec8-cohesin complexes are cleaved by separase in two steps. First, cohesin on chromosome arms, but not that at pericentromeres, is cleaved at anaphase I onset, resolving chiasmata and triggering segregation of homologous chromosomes. Second, at anaphase II, cohesin

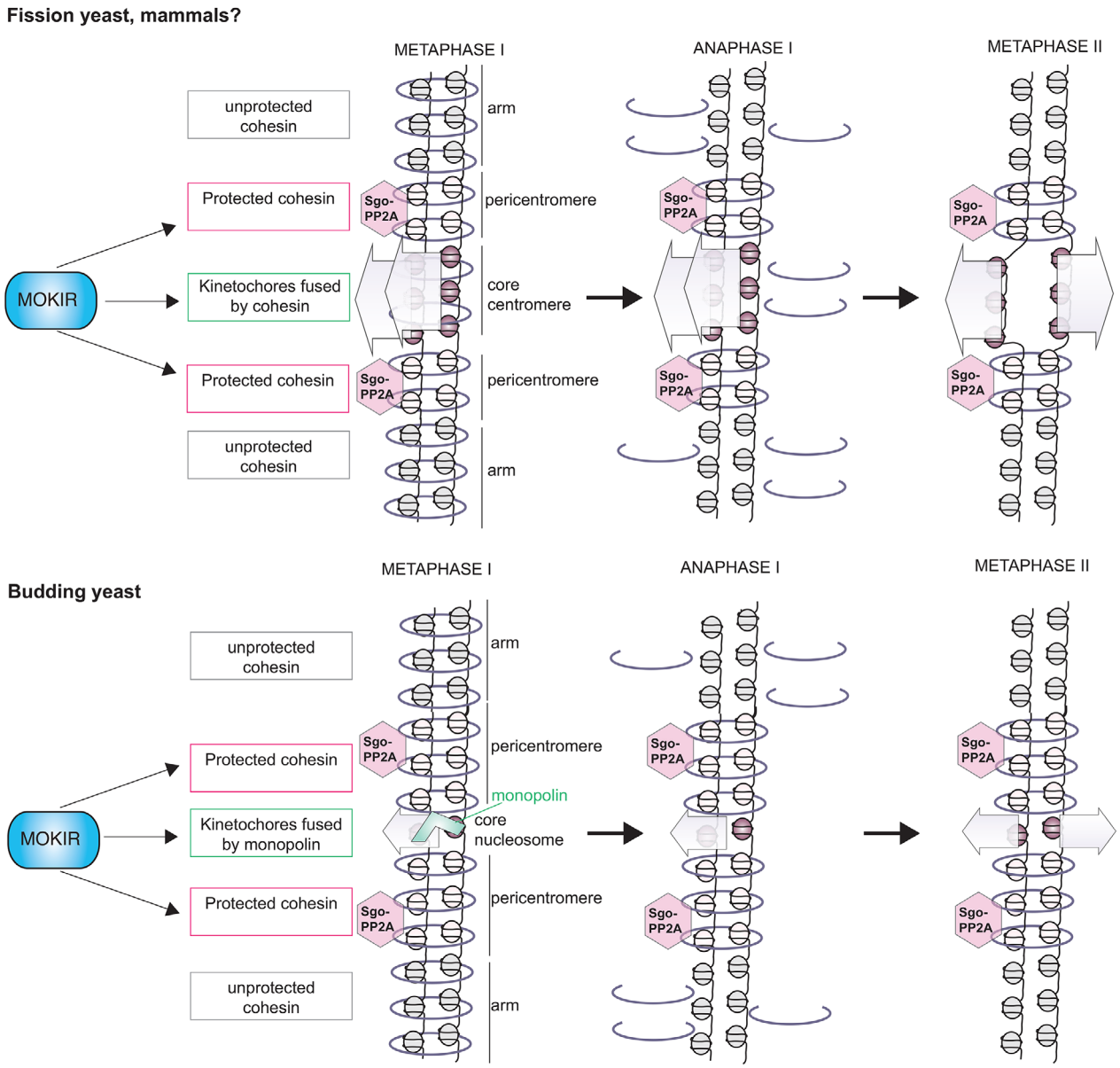



**Figure 3.** Model for distinct regulation of centromeric domains by MOKIRs. In fission yeast, the centromeric region is organized such that the core centromere, where the kinetochores assemble, is flanked by surrounding heterochromatin that is cohesin-rich. During meiosis I, Rec8-cohesin is established within the core centromere and builds cohesive links in a Moa1$^{MOKIR}$-dependent manner. This directs sister kinetochores to attach to microtubules from the same pole. Gray-shaded arrows represent the orientation of microtubule attachments. In schematics showing metaphase I and anaphase I, double arrows indicate co-orientation by centromere cohesion and a single arrow indicates monopolin-based sister kinetochore fusion. Sister kinetochore mono-orientation relies on the recruitment of Plo1$^{Polo}$ kinase to kinetochores; however, the relevant substrates are not known. Meanwhile, Moa1$^{MOKIR}$ promotes the maintenance of cohesin within the pericentromere until meiosis II, potentially in part by ensuring the retention of shugoshin-PP2A within the pericentromeric domain, where cohesin is protected. At anaphase I onset, shugoshin protects a specific centromeric pool of cohesin from cleavage, while cohesin cleavage on arms triggers homologous chromosome segregation. Cohesin cleavage in the core centromere has also been proposed to reverse mono-orientation, so that sister kinetochores bi-orient in meiosis II. In budding yeast, the process is similar except that monopolin, rather than cohesin, directs sister kinetochore mono-orientation. In *Drosophila*, ectopic activity of Polo in *mtrm* mutants promotes cohesion loss but it is not yet known which domains of cohesin are regulated by Mtrm$^{MOKIR}$ and what its impact on sister kinetochore mono-orientation is.

persisting at pericentromeres is cleaved to trigger the segregation of sister chromatids.

Cohesin cleavage requires the prior phosphorylation of its Rec8 kleisin subunit.[26] Full Rec8 phosphorylation relies on PLK1$^{Polo}$ in mice,[27] Cki1 in fission yeast,[28] and casein kinase 1$\delta$ (CK1$\delta$), Dbf4-dependent kinase (DDK) and Cdc5$^{Polo}$ in budding yeast,[26,29] although the contribution of Cdc5$^{Polo}$ to cleavage is under debate.[26,29,30] However, our recent data has shown that tethering of Cdc5$^{Polo}$ directly to Rec8 in meiosis can promote loss of cohesion between sister chromatids at anaphase I, suggesting that Cdc5$^{Polo}$ contributes to cohesin removal,[12] although whether it does so through promoting separase-dependent

Rec8 cleavage or via the cleavage-independent, Wapl-dependent cohesin removal pathway is unknown. In *Drosophila*, meiotic cohesin does not contain a Rec8-like subunit. Instead, a meiosis-specific complex containing SMC1 and SMC3 together with the SOLO and SUNN proteins localizes to centromeres, where it persists until metaphase II.[31–33] However, little is known about the phosphorylation state of SOLO-SUNN cohesin in meiosis, and how its removal from chromosomes is regulated.

Protection of pericentromeric cohesin is brought about by the pericentromeric adaptor protein Shugoshin (Table 1). Shugoshin specifically localizes to pericentromeres by binding histone H2A that has been phosphorylated by Bub1.[34–39] In mitosis, phosphorylation of human Sgo1 by CDKs promotes its binding to cohesin, and this is required to protect pericentromeric cohesin from its non-proteolytic removal by Wapl.[39,40] Shugoshins also bind to and localize with cohesin during meiosis.[12,41] Whether shugoshins also counteract Wapl during meiosis is not clear, but they are known to protect Rec8 from separase-dependent cleavage in a multitude of organisms, thereby ensuring the maintenance of pericentromeric cohesion until meiosis II.[41–47] To achieve this, shugoshins recruit protein phosphatase 2A (PP2A; Table 1) to pericentromeres by binding of the PP2A B' regulatory subunit.[48–51] There, PP2A is thought to dephosphorylate cohesin, thus preventing its cleavage by separase. Artificial tethering of PP2A to arm cohesin in fission yeast[51] or budding yeast[52] impairs cohesin cleavage on chromosome arms, highlighting the importance of restricting PP2A activity to the pericentromere. Thus, cohesin protection in the pericentromere requires the careful balancing of cohesin-directed kinase and phosphatase activity; disruption of this balance is likely to interfere with the maintenance of pericentromeric cohesin after metaphase I.

### 3.2. MOKIRs Protect Cohesin by Regulating Shugoshin and the Activity of Cohesin Kinases

MOKIRs are required for the retention of centromeric cohesion in meiosis I in budding yeast, fission yeast, *Drosophila* and mouse, and may contribute in several different ways (**Figure 4**).[15,16,53–56] Initial reports of Spo13$^{MOKIR}$'s involvement in this process suggested that it is required for the maintenance of Sgo1 at budding yeast pericentromeres during metaphase I.[36] Similarly, mouse Sgo2 localization to pericentromeres is reportedly reduced in *Meikin*$^{-/-}$ mutants.[15] In fission yeast, however, Sgo1 levels are unaltered in *moa1*Δ cells.[56] It has been proposed that Moa1$^{MOKIR}$-Plo1$^{Polo}$ regulates centromeric Bub1 localization together with the spindle checkpoint kinase Mph1$^{Mps1}$, since *moa1*Δ *mph1*Δ double mutants show additive reductions in Bub1 de-localization, and consequent loss of Sgo1 localization.[56] Still, the fact that *moa1*Δ cells show meiotic cohesion defects despite correctly localizing Sgo1 suggests that mechanisms other than Bub1 de-localization drive cohesion loss in *moa1*Δ cells.

Similar to fission yeast, our recent evidence in budding yeast shows that Spo13$^{MOKIR}$-Cdc5$^{Polo}$ promotes cohesin protection, since artificial tethering of Cdc5$^{Polo}$ to kinetochores, but not to cohesin, mildly enhanced cohesin retention in *spo13*Δ anaphase I cells, although this was not sufficient to provide sister chromatid cohesion.[12] Contrary to initial findings, it is now established that the cohesin protector Sgo1-PP2A is appropriately localized in metaphase I *spo13*Δ cells.[12,36,54] Instead, over-activity of the kinases (CK1*δ* and DDK) that phosphorylate cohesin to promote its cleavage may explain defective cohesion in the absence of Spo13$^{MOKIR}$, because inhibition of either one of the redundant cohesin kinases CK1*δ* and DDK in *spo13*Δ cells is sufficient to prevent sister chromatid segregation in anaphase I.[12] Similarly, depletion of Cdc5$^{Polo}$ prevents sister chromatid segregation in *spo13*Δ, though the molecular reasons remain unclear.[12] Importantly, Spo13$^{MOKIR}$ binds all three cohesin kinases directly[11,12] or indirectly,[57] suggesting that it may restrict their activity through direct interactions. Although MOKIRs are not required for establishing Sgo1 localization at pericentromeres in meiosis I, at least in budding or fission yeast, MOKIR-mediated kinase suppression may be important to maintain Sgo1 localization, because CK1*δ* promotes the permanent removal of Sgo1 upon anaphase I onset in *spo13*Δ cells, in addition to its function in cohesin phosphorylation.[12] Surprisingly, although artificial tethering of Sgo1 to cohesin reinstates centromeric cohesin in *spo13*Δ mutants, sister chromatids nevertheless segregate upon anaphase I onset.[12] This is analogous to findings in fission yeast, where loss of Moa1$^{MOKIR}$ interferes with cohesin's ability to link sister chromatids in the core centromere for sister kinetochore mono-orientation (see below).[58] Thus, it is tempting to speculate that the apparent over-activity of kinases disturbs the linkage of sister chromatids in the pericentromere of meiotic budding yeast and fission yeast cells. As described below, MOKIRs in fission yeast and mice are thought to direct the formation of inter-sister cohesive linkages within the core centromere to direct sister kinetochore mono-orientation. It is conceivable that Spo13$^{MOKIR}$ plays a similar role in budding yeast, except in this case the cohesive linkages would be in the pericentromere and represent the key linkages that should be protected until meiosis II (Figure 3). Further experiments will, however, be required to ascertain if this is the case and, if so, whether a similar underlying mechanism is at play in both organisms.

Recent evidence from *Drosophila* supports the notion that kinase inhibition may similarly be the critical function of Mtrm$^{MOKIR}$ in cohesin protection. However, in contrast to other organisms, where MOKIRs appear to target Polo to kinetochores (see below), *Drosophila* Mtrm$^{MOKIR}$ may sequester Polo *away* from chromosomes. In *mtrm* null oocytes or oocytes carrying a mutation in the PBD binding region, active Polo kinase is released from the spindle and increased amounts are found on DNA.[16] Since defective centromeric cohesion in *mtrm* null oocytes is rescued by lowering Polo activity,[16,59] Mtrm$^{MOKIR}$ must preserve cohesion by sequestration of Polo on the spindle. How ectopic Polo triggers cohesion loss is not clear, since the cohesin protector MEI-S332$^{Sgo}$ (which is related to the later-discovered shugoshins)[41,47,60] is appropriately localized in metaphase I,[16] similar to *spo13*Δ and *moa1*Δ mutants in budding yeast and fission yeast, respectively. Instead, Mtrm$^{MOKIR}$ may interfere with Polo's ability to phosphorylate proteins promoting cohesin cleavage. Alternatively, Mtrm$^{MOKIR}$ may prevent the premature removal of MEI-S332$^{Sgo}$ in anaphase I by counteracting Polo, which is known to delocalize MEI-S332$^{Sgo}$ from chromosomes in meiosis II.[61] This hypothesis is particularly attractive because such a function of Mtrm$^{MOKIR}$ would mirror the role of budding yeast Spo13$^{MOKIR}$ in preventing the CK1*δ*-mediated removal of Sgo1 in anaphase I.

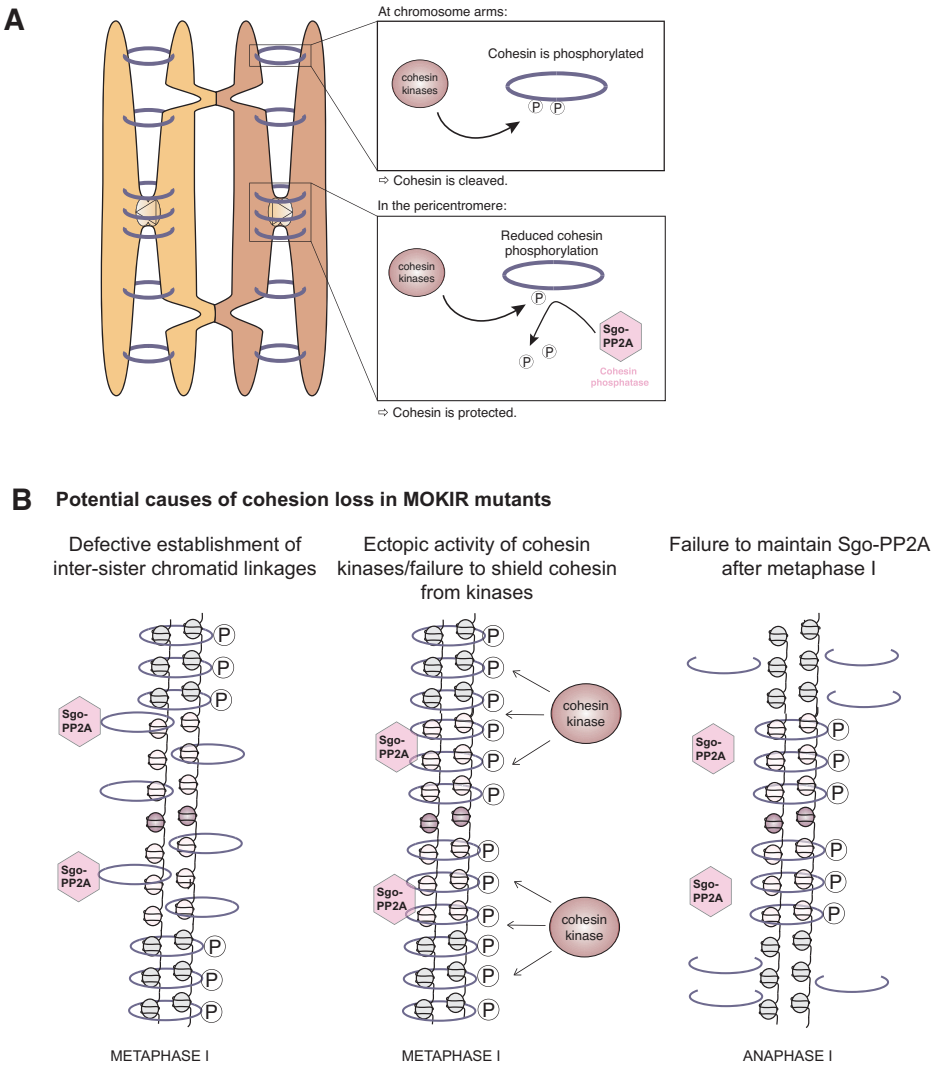



**Figure 4.** Potential causes of cohesin loss in MOKIR mutants. A) Cohesin cleavage requires its prior phosphorylation by cohesin kinases. Although phosphorylation occurs along the length of the chromosome, it is counteracted in the pericentromere by PP2A, which is recruited by the action of the pericentromeric adaptor protein Shugoshin (Sgo1 in yeast). Reduced cohesin phosphorylation in the pericentromere prevents its cleavage by separase in anaphase I. B) MOKIRs might assist Sgo1-mediated cohesin protection in a number of ways: first, they could ensure the building of inter-sister cohesion; second, they could shield cohesin from the activity of cohesin kinases; and third, MOKIRs may act to retain shugoshin-mediated protection until metaphase II.

In summary, regulation of Polo kinase by MOKIRs aids pericentromeric cohesin retention in meiosis I, but other mechanisms are likely to contribute. Indeed, as for the budding yeast example, MOKIRs may regulate other kinases. How ectopic kinase activity results in the premature cleavage of pericentromeric cohesin is not completely clear. However, future studies should address if MOKIRs and the kinases they regulate maintain centromeric cohesion beyond meiosis I through mechanisms involving: 1) shugoshin persistence at centromeres during/beyond anaphase I, 2) shielding cohesin from cleavage-promoting phosphorylation by dis-regulated kinases, and 3) altering the types and positions of cohesin-dependent linkages that are established within core centromeres (Figure 2D, Figure 3). These mechanisms are not mutually exclusive and both inhibitory and activating interactions can be envisaged.

## 4. MOKIRs Promote Mono-Orientation by Regulating Polo Kinases

### 4.1. Different Organisms have Distinct Requirements for Sister Kinetochore Mono-orientation

To segregate homologous chromosomes in meiosis I, sister kinetochores need to face the same pole, a property referred to as mono-orientation. In budding yeast, *Drosophila*, maize and mouse, the fusion of sister kinetochores into a single microtubule-binding entity appears to underlie their mono-orientation during meiosis I.[15,62–65] MOKIRs have been implicated in this process in mouse, but it is best understood in budding and fission yeast, where distinct mechanisms have emerged (Figures 3 and 5).

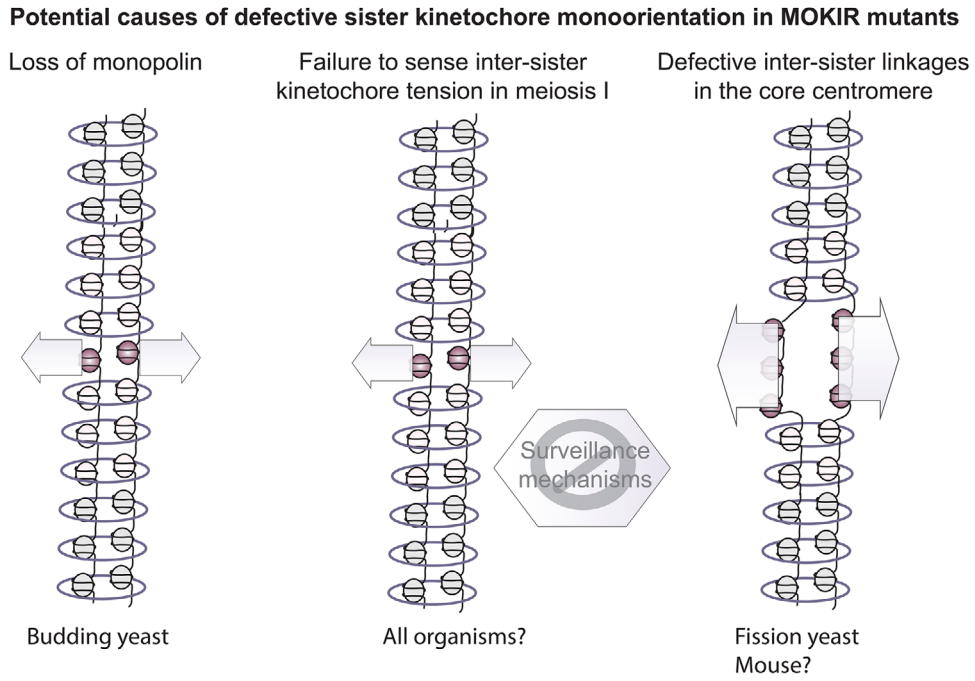



**Figure 5.** Regulation of mono-orientation by MOKIRs. The conserved function of MOKIRs is to recruit Polo kinase to kinetochores, and this is important for sister kinetochore mono-orientation in budding yeast, fission yeast and mouse. However, the exact function and targets of Polo have so far remained elusive. In budding yeast, cohesin is largely dispensable for mono-orientation and monopolin is required instead. Consistently, artificial tethering of $Cdc5^{Polo}$ to kinetochores, but not cohesin, forces sister kinetochore co-orientation, even in the absence of monopolin. Thus, a component of the kinetochore, or a kinetochore-associated protein, is the likely target of $Cdc5^{Polo}$ for mono-orientation. Potentially, $Cdc5^{Polo}$ alters surveillance mechanisms to ensure sister kinetochore mono-orientation, rather than biorientation. In fission yeast and mouse, Polo is likely to target cohesin, since core cohesion is typically lost in the absence of the respective MOKIR; however, the evidence for centromeric cohesion loss is currently strongest in fission yeast. Whether *Drosophila* Mtrm also regulates mono-orientation is currently unclear.

### 4.2. Cohesin is Important for Sister Kinetochore Mono-orientation in Fission Yeast

Fission yeast centromeres comprise a central core, where kinetochores assemble, together with flanking pericentromeric heterochromatin, where cohesin is highly enriched and protected during meiosis I. Mono-orientation requires sister chromatid cohesion in the core centromere. This is brought about by cohesin complexes containing the meiosis-specific kleisin subunit Rec8,[66] which are specifically enriched in this region.[58,67] In the absence of Rec8, cohesin containing the alternative kleisin subunit $Rad21^{Scc1}$, which is typically restricted to the pericentromere, moves into the core centromere; however, mono-orientation is still defective,[68] suggesting a particular requirement for Rec8 in this process. A screen for mono-orientation-defective mutants identified several cohesin regulators, in addition to $Moa1^{MOKIR}$, which was found to specifically regulate core centromere cohesion.[10,58,68,69] $Moa1^{MOKIR}$ interacts with $Plo1^{Polo}$ and recruits it to kinetochores, and $Moa1^{MOKIR}$ mutants unable to bind $Plo1^{Polo}$ are defective for mono-orientation.[15,56] Although the target of $Plo1^{Polo}$ in mono-orientation is still unknown, cohesin is a potential candidate. In *moa1*Δ mutants, defective mono-orientation can be alleviated by artificially linking the centromere sequences of sister chromatids.[58] Thus, in fission yeast, cohesin appears to promote mono-orientation by bringing sister core centromere sequences in close proximity, and this function may require $Moa1^{MOKIR}$-dependent kinetochore recruitment of $Plo1^{Polo}$. Recent work has suggested that cohesin in the core centromere also directs mono-orientation in mouse.[70] Interestingly, this work argued for a role of separase-dependent cleavage of cohesin in the destruction of both mono-orientation and cohesin protection after meiosis I.[70] Collectively, these findings lead to the speculation that the crucial activity of fission yeast and mouse MOKIR-Polo complexes in both mono-orientation and cohesin protection might be to alter core cohesin to make it refractory to separase-dependent cleavage.

### 4.3. Monopolin Directs Sister Kinetochore Mono-orientation in Budding Yeast

In contrast, evidence suggests that sister kinetochore mono-orientation is achieved independently of Rec8 cohesin in budding yeast. Replacement of the meiosis-specific cohesin subunit Rec8 with its mitotic counterpart, Scc1, does not affect mono-orientation[71] and deletion of *REC8* causes only a minor mono-orientation phenotype.[72] In budding yeast, mono-orientation is established as a result of sister kinetochore fusion by a dedicated protein complex, called monopolin.[63] Monopolin consists of four subunits: the meiosis-specific kinetochore protein Mam1,[71] the nucleolar proteins Lrs4 and Csm1,[73] and CK1$\delta$.[74] The monopolin complex forms a V-shaped homodimer,[75] in which the Csm1 subunits directly interact with the kinetochore protein

Dsn1, presumably on sister kinetochores.[75–78] This is thought to fuse sister kinetochores to create a common microtubule-interaction surface.[63,75] Monopolin-induced mono-orientation requires the action of a number of different kinases. First, monopolin-associated CK1$\delta$ brings the monopolin complex to kinetochores and CK1$\delta$ kinase activity is required to prevent sister kinetochores splitting in metaphase I.[74] Surprisingly, however, sister chromatid co-segregation in anaphase I is only modestly perturbed when CK1$\delta$ is inhibited[12] and CK1$\delta$ activity is dispensable for kinetochore fusion in vitro.[63] Thus, the function of CK1$\delta$ in mono-orientation is still largely elusive. Second, DDK is required for monopolin association with kinetochores.[11] Third, Cdc5$^{Polo}$ activity is required for the release of the monopolin subunits Csm1 and Lrs4 from the nucleolus, [79] thus promoting monopolin assembly at kinetochores.[73] Lastly, phosphorylation sites within the monopolin-binding region of Dsn1 that are the targets of CK1$\delta$ in vitro[80] are also required for mono-orientation, although it is unknown whether this is the kinase responsible for Dsn1 phosphorylation in vivo.[78]

Although budding yeast apparently uses a monopolin- rather than cohesin-dependent mechanism to direct mono-orientation, a role for Spo13$^{MOKIR}$ is well established.[54,55] Spo13$^{MOKIR}$ interacts with Cdc5$^{Polo}$ and recruits it to kinetochores, and this interaction promotes monopolin recruitment and mono-orientation in metaphase I-arrested cells. However, a mutant of Spo13$^{MOKIR}$ in which the Cdc5$^{Polo}$ interaction site is mutated still largely co-segregates sister chromatids in anaphase I,[12] possibly due to residual Spo13$^{MOKIR}$-Cdc5$^{Polo}$ interaction in this mutant.[11] Artificial tethering of Cdc5$^{Polo}$ to kinetochores restores co-segregation of sister chromatids in cells lacking Spo13$^{MOKIR}$ and, remarkably, this overrides the requirement of monopolin for mono-orientation.[12] Thus, forcing Cdc5$^{Polo}$ localization to kinetochores likely does not induce bona fide mono-orientation in budding yeast, although the resulting molecular setup is strikingly similar to that of fission yeast and mouse (see below). Consistently, although Spo13$^{MOKIR}$ is essential for monopolin recruitment,[12,54,55] tethering Cdc5$^{Polo}$ to kinetochores in the absence of Spo13$^{MOKIR}$ does not restore monopolin localization. This suggests that Spo13$^{MOKIR}$ directs monopolin association with kinetochores independently of Cdc5$^{Polo}$.[12] Furthermore, unlike tethering of Cdc5$^{Polo}$ to kinetochores, its tethering to cohesin does not rescue the mono-orientation defect of *spo13*Δ cells,[12] suggesting that the crucial target of Cdc5$^{Polo}$ for mono-orientation in budding yeast may be a kinetochore component. Nevertheless, the finding that Cdc5$^{Polo}$ tethering to kinetochores can direct sister chromatid co-segregation independently of monopolin hints at a conserved ancestral mechanism for kinetochore-associated Polo kinases in defining reductional meiosis I segregation.

### 4.4. MOKIRs Play Poorly-Understood Roles in Sister Kinetochore Mono-orientation

Outside the yeasts, the mechanism of sister kinetochore mono-orientation and role for MOKIRs is even less clear. In mouse, MEIKIN$^{MOKIR}$ has been shown to be a key regulator of mono-orientation.[15] Although the underlying mechanisms of MEIKIN$^{MOKIR}$-mediated mono-orientation are poorly understood, MEIKIN$^{MOKIR}$ recruits PLK1$^{Polo}$, whose activity is required for mono-orientation, to kinetochores in meiosis I.[15] Notably, both Moa1$^{MOKIR}$ and MEIKIN$^{MOKIR}$ bind to kinetochores via the CENP-C kinetochore subunit, providing further evidence to the notion that these proteins are functionally conserved.[15]

In *Drosophila*, sister centromere fusion similar to that seen in budding yeast has been proposed as a mechanism of mono-orientation.[62] Fusion depends on both centromere cohesion and the activity of protein phosphatase 1, which is thought to counteract the stabilization of kinetochore-microtubule interactions by Polo and BubR1 kinases.[81] Thus, it appears that, in contrast to other model organisms, *Drosophila* Polo counteracts sister centromere fusion. Whether Mtrm$^{MOKIR}$ is important for mono-orientation is currently unresolved, though current findings do not rule this possibility out. Homozygous or heterozygous *mtrm* mutants precociously separate sister centromeres and deletion of a single allele of Polo in *mtrm* heterozygotes rescues this phenotype,[16] suggesting that Mtrm$^{MOKIR}$ primarily acts to counteract the activity of Polo. Indeed, catalytically active Polo relocalizes from the spindle onto the DNA in *mtrm* null mutants, thereby likely altering the phosphorylation state of Polo targets on chromosomes and the spindle. Although *mtrm* mutants display a loss of centromere fusion, this is most likely caused by defective centromeric cohesion.[16] As a consequence, it is currently difficult to assess the status of sister kinetochore orientation in *mtrm* mutants, thus precluding any conclusions about the mono-orientation functions of Mtrm$^{MOKIR}$ and the pool of Polo that it regulates.

Collectively, the above findings argue that the conserved function of MOKIRs is to regulate Polo kinases, either by altering Polo activity or localization. The MOKIRs Spo13, Moa1 and MEIKIN recruit Polo to meiosis I kinetochores, where it promotes mono-orientation through mechanisms that are likely to be species-specific, whereas *Drosophila* Mtrm$^{MOKIR}$ appears to exclude Polo from chromosomes. Cohesin may be the conserved Polo target in organisms that do not contain monopolin, whereas budding yeast Cdc5$^{Polo}$ may target a kinetochore protein or, potentially, the checkpoint and error correction machinery. Understanding the differences between monopolin-dependent and monopolin-independent mechanisms of mono-orientation may help identify MOKIR-Polo targets in various organisms. Perhaps, monopolin-mediated mono-orientation is not feasible in organisms in which centromeric domains are larger than the budding yeast point centromere and in which more than one microtubule attaches to a single kinetochore. Therefore, the MOKIR-Polo system may have been modified throughout the course of evolution to facilitate cohesin-mediated centromere fusion as a substitute for monopolin-dependent mono-orientation.

## 5. MOKIRs Perform Specialized Functions in Budding Yeast and *Drosophila*

Given the strong sequence divergence of MOKIRs, it is not surprising that specialized functions have been reported for a number of these proteins. *spo13*Δ mutants, for example, only undergo a single meiotic division[82] characterized by a mixture of reductional and equational chromosome segregation.[52,53] This

phenotype is not seen in fission yeast *moa1*Δ cells[10] or mouse *Meikin*$^{-/-}$ oocytes.[15] Little is known about the molecular basis of the altered cell cycle in *spo13*Δ cells, but it has been observed that deletion of the spindle checkpoint component *MAD2* restores the second division in a majority of *spo13*Δ cells,[53] although chromosome segregation is still defective in *spo13*Δ *mad2*Δ strains.[52] How *MAD2* deletion promotes a second division in the absence of Spo13$^{MOKIR}$ is unknown, but it has been proposed that the spindle checkpoint-dependent metaphase I delay observed in *spo13*Δ mutants might cause cells to run out of time to perform a second division.[53] However, monopolin mutants undergo a similar metaphase I delay to *spo13*Δ cells, but nonetheless appear to biochemically undergo two meiotic divisions and largely form four spores,[54,55,71] arguing against the notion that *spo13*Δ cells do not have enough time to complete two divisions. Instead, it seems more likely that *spo13*Δ mutants activate the meiotic exit program already after meiosis I. One of the key characteristics of meiotic exit is the accumulation of the meiosis-specific APC/C activator Ama1, which degrades key meiotic division proteins such as Cdc5$^{Polo}$ and the meiosis-specific transcription factor Ndt80.[3] Indeed, deletion of Ama1 has been shown to partially restore meiosis II spindle formation in *spo13*Δ cells,[55] suggesting that premature Ama1 activation might contribute to early meiotic exit upon Spo13$^{MOKIR}$ loss. Interestingly, the accumulation of Ama1 and a variety of other events occurring during meiotic exit in wild-type cells depends on the activity of CK1*δ*.[83] Given our findings that Spo13$^{MOKIR}$ restricts CK1*δ* activity to promote cohesin protection during meiosis I,[12] we speculate that a similar activity of Spo13$^{MOKIR}$ prevents premature meiotic exit.

Although Spo13$^{MOKIR}$ and Mtrm$^{MOKIR}$ share a similar LEN degradation motif (Figure 2C), their degradation occurs at different times. Spo13$^{MOKIR}$ is degraded at the onset of anaphase I,[17] but Mtrm$^{MOKIR}$ persists until the end of meiosis when it is targeted for proteasomal destruction by the meiosis-specific APC/C$^{Cort}$ form.[18] This ensures sufficient Polo activity at the end of meiosis to drive the oocyte-to-embryo transition.[18] Additionally, the inhibitory action of Mtrm$^{MOKIR}$ toward Polo is also required at the onset of meiosis, where it ensures that the G2 arrest preceding meiotic entry in oocytes is maintained.[59] In other organisms, pre-division functions of MOKIRs are not known, but, at least in budding yeast, may not be required because Cdc5$^{Polo}$ activity is restricted prior to metaphase I due to active degradation[3] and lack of transcription.[84]

In summary, few specialized functions of MOKIRs, in addition to promoting mono-orientation and cohesin protection, are known, and the mechanisms governing these functions are poorly understood. However, in analogy to the potential function of MOKIRs in cohesin protection, these proteins may control additional meiotic processes through a general property of spatiotemporally restricting the function of meiotic kinases.

## 6. Conclusions and Future Perspectives

MOKIRs are key meiotic proteins that almost single-handedly appear to convert many aspects of mitotic chromosome segregation into essential adaptations for meiosis. Yet, their structural features and mechanistic functions are largely elusive. The common denominator for these proteins appears to be their ability to bind and spatially regulate Polo kinases, thus promoting sister kinetochore mono-orientation in meiosis I. Beyond this, MOKIRs seem to act by restricting the activity of meiotic kinases. Although it is tempting to speculate that these proteins directly inhibit meiotic kinases, as has been suggested in the case of Mtrm$^{MOKIR}$ and Polo, it is crucial that these kinases still retain some activity in the presence of MOKIRs to perform their essential meiotic functions. One possible explanation to this conundrum is that MOKIRs may target specific pools of a particular kinase, as has been shown recently for Mtrm$^{MOKIR}$.[16] Analysis of the role of post-translational modifications in the regulation of MOKIRs may provide some clues as to how the diverse functions of these proteins are integrated. Moreover, the identification of separation-of-function mutants would greatly aid the study of MOKIRs. It is also essential to determine how MOKIRs are deactivated in meiosis II. Although Spo13$^{MOKIR}$ is degraded in anaphase I, spore viability is similar to wild type in a mutant resistant to degradation,[17] suggesting additional deactivation mechanisms. However, to fully understand the multifaceted functions of MOKIRs, a greater understanding of the meiotic chromosome segregation adaptations in general is required, in particular with regard to the mechanisms of mono-orientation and cohesin regulation. Research on MOKIRs so far has identified them as key rulers of meiotic kinases. The next challenge is to elucidate the identity and role of phosphorylation events during meiotic chromosome segregation and how unruly kinases in MOKIR-deficient cells affect the balance of post-translational modifications in the meiotic cell cycle.

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## Conflict of Interest

The authors declare no conflict of interest.

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[1] G. Cabral, A. Marques, V. Schubert, A. Pedrosa-Harand, P. Schlögelhofer, *Nat. Commun.* **2014**, *5*, 5070.
[2] A. L. Marston, A. Amon, *Nat. Rev. Mol. Cell Biol.* **2004**, *5*, 983.
[3] E. Okaz, O. Argüello-Miranda, A. Bogdanova, P. K. Vinod, J. J. Lipp, Z. Markova, I. Zagoriy, B. Novak, W. Zachariae, *Cell* **2012**, *151*, 603.
[4] A. L. Marston, K. Wassmann, *Front Cell Develop Biol* **2017**, *5*, 109.

 

[5] F. Sievers, A. Wilm, D. Dineen, T. J. Gibson, K. Karplus, W. Li, R. Lopez, H. McWilliam, M. Remmert, J. Söding, J. D. Thompson, D. G. Higgins, *Molecular Systems Biology* **2011**, *7*, 539.
[6] L. A. Kelley, S. Mezulis, C. M. Yates, M. N. Wass, M. J. E. Sternberg, *Nat. Protoc.* **2015**, *10*, 845.
[7] A. M. Bonner, S. E. Hughes, J. A. Chisholm, S. K. Smith, B. D. Slaughter, J. R. Unruh, K. A. Collins, J. M. Friederichs, L. Florens, S. K. Swanson, M. C. Pelot, D. E. Miller, M. P. Washburn, S. L. Jaspersen, R. S. Hawley, *Proc. Natl. Acad. Sci. USA* **2013**, *110*, E1222.
[8] A. M. Bonner, R. S. Hawley, *Mol. Biol. Evol.* **2019**, *36*, 69.
[9] P. E. Wright, H. J. Dyson, *Nat. Rev. Mol. Cell Biol.* **2015**, *16*, 18.
[10] S. Yokobayashi, Y. Watanabe, *Cell* **2005**, *123*, 803.
[11] J. Matos, J. J. Lipp, A. Bogdanova, S. Guillot, E. Okaz, M. Junqueira, A. Shevchenko, W. Zachariae, *Cell* **2008**, *135*, 662.
[12] S. Galander, R. E. Barton, W. E. Borek, C. Spanos, D. A. Kelly, D. Robertson, J. Rappsilber, A. L. Marston, *Dev. Cell* **2019**, *49*, 526.
[13] A. E. H. Elia, L. C. Cantley, M. B. Yaffe, *Science* **2003**, *299*, 1228.
[14] A. E. H. Elia, P. Rellos, L. F. Haire, J. W. Chao, F. J. Ivins, K. Hoepker, D. Mohammad, L. C. Cantley, S. J. Smerdon, M. B. Yaffe, *Cell* **2003**, *115*, 83.
[15] J. Kim, K.-I. Ishiguro, A. Nambu, B. Akiyoshi, S. Yokobayashi, A. Kagami, T. Ishiguro, A. M. Pendás, N. Takeda, Y. Sakakibara, T. S. Kitajima, Y. Tanno, T. Sakuno, Y. Watanabe, *Nature* **2015**, *517*, 466.
[16] A. M. Bonner, S. E. Hughes, R. S. Hawley, *Curr. Biol.* **2020**, 1.
[17] M. Sullivan, D. O. Morgan, *J. Biol. Chem.* **2007**, *282*, 19710.
[18] Z. J. Whitfield, J. Chisholm, R. S. Hawley, T. L. Orr-Weaver, *PLoS Biol.* **2013**, *11*, e1001648.
[19] K. Nasmyth, C. H. Haering, *Annu. Rev. Genet.* **2009**, *43*, 525.
[20] S. Yatskevich, J. Rhodes, K. Nasmyth, *Annu. Rev. Genet.* **2019**, *53*, 445.
[21] K. Challa, M.-S. Lee, M. Shinohara, K. P. Kim, A. Shinohara, *Nucleic Acids Res.* **2016**, gkw034.
[22] K. Challa, G. Fajish, V. M. Shinohara, F. Klein, S. M. Gasser, A. Shinohara, *PLoS Genet.* **2019**, *15*, e1007851.
[23] O. Crawley, C. Barroso, S. Testori, N. Ferrandiz, N. Silva, M. Castellano-Pozo, A. L. Jaso-Tamame, E. Martinez-Perez, *eLife* **2016**, *5*, 563.
[24] M. C. C. Silva, S. Powell, S. Ladstätter, J. Gassler, R. Stocsits, A. Tedeschi, J.-M. Peters, K. Tachibana, *J. Cell Biol.* **2020**, *219*, 977.
[25] K. Tachibana-Konwalski, J. Godwin, L. van der Weyden, L. Champion, N. R. Kudo, D. J. Adams, K. Nasmyth, *Genes Dev.* **2010**, *24*, 2505.
[26] G. A. Brar, B. M. Kiburz, Y. Zhang, J.-E. Kim, F. White, A. Amon, *Nature* **2006**, *441*, 532.
[27] N. R. Kudo, M. Anger, A. H. F. M. Peters, O. Stemmann, H.-C. Theussl, W. Helmhart, H. Kudo, C. Heyting, K. Nasmyth, *J. Cell Sci.* **2009**, *122*, 2686.
[28] T. Ishiguro, K. Tanaka, T. Sakuno, Y. Watanabe, *Nat. Cell Biol.* **2010**, *12*, 500.
[29] V. L. Katis, J. J. Lipp, R. Imre, A. Bogdanova, E. Okaz, B. Habermann, K. Mechtler, K. Nasmyth, W. Zachariae, *Dev. Cell* **2010**, *18*, 397.
[30] M. A. Attner, M. P. Miller, L.-S. Ee, S. K. Elkin, A. Amon, *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 14278.
[31] R. S. Khetani, S. E. Bickel, *J. Cell Sci.* **2007**, *120*, 3123.
[32] B. Krishnan, S. E. Thomas, R. Yan, H. Yamada, I. B. Zhulin, B. D. McKee, *Genetics* **2014**, *198*, 947.
[33] R. Yan, S. E. Thomas, J.-H. Tsai, Y. Yamada, B. D. McKee, *J. Cell Biol.* **2010**, *188*, 335.
[34] Z. Tang, Y. Sun, S. E. Harley, H. Zou, H. Yu, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 18012.
[35] T. S. Kitajima, S. Hauf, M. Ohsugi, T. Yamamoto, Y. Watanabe, *Curr. Biol.* **2005**, *15*, 353.
[36] B. M. Kiburz, D. B. Reynolds, P. C. Megee, A. L. Marston, B. Lee, T. I. Lee, S. S. Levine, R. A. Young, A. Amon, *Genes Dev.* **2005**, *19*, 3017.
[37] J. Fernius, K. G. Hardwick, *PLoS Genet.* **2007**, *3*, e213.
[38] S. A. Kawashima, Y. Yamagishi, T. Honda, K.-I. Ishiguro, Y. Watanabe, *Science* **2010**, *327*, 172.
[39] H. Liu, L. Jia, H. Yu, *Curr. Biol.* **2013**, *23*, 1927.
[40] H. Liu, S. Rankin, H. Yu, *Nat. Cell Biol.* **2013**, *15*, 40.
[41] T. S. Kitajima, S. A. Kawashima, Y. Watanabe, *Nature* **2004**, *427*, 510.
[42] A. L. Marston, W.-H. Tham, H. Shah, A. Amon, *Science* **2004**, *303*, 1367.
[43] V. L. Katis, M. Gálová, K. P. Rabitsch, J. Gregan, K. Nasmyth, *Curr. Biol.* **2004**, *14*, 560.
[44] E. Llano, R. Gómez, C. Gutiérrez-Caballero, Y. Herrán, M. Sánchez-Martín, L. Vázquez-Quiñones, T. Hernández, E. de Alava, A. Cuadrado, J. L. Barbero, J. A. Suja, A. M. Pendás, *Genes Dev.* **2008**, *22*, 2400.
[45] J. Lee, T. S. Kitajima, Y. Tanno, K. Yoshida, T. Morita, T. Miyano, M. Miyake, Y. Watanabe, *Nat. Cell Biol.* **2008**, *10*, 42.
[46] A. Rattani, M. Wolna, M. Ploquin, W. Helmhart, S. Morrone, B. Mayer, J. Godwin, W. Xu, O. Stemmann, A. Pendas, K. Nasmyth, *eLife* **2013**, *2*, https://doi.org/10.7554/eLife.01133.
[47] A. W. Kerrebrock, W. Y. Miyazaki, D. Birnby, T. L. Orr-Weaver, *Genetics* **1992**, *130*, 827.
[48] T. S. Kitajima, T. Sakuno, K.-I. Ishiguro, S.-I. Iemura, T. Natsume, S. A. Kawashima, Y. Watanabe, *Nature* **2006**, *441*, 46.
[49] B. S. Pinto, T. L. Orr-Weaver, *Proc. Natl. Acad. Sci. USA* **2017**, *114*, 12988.
[50] Z. Xu, B. Cetin, M. Anger, U. S. Cho, W. Helmhart, K. Nasmyth, W. Xu, *Mol. Cell* **2009**, *35*, 426.
[51] C. G. Riedel, V. L. Katis, Y. Katou, S. Mori, T. Itoh, W. Helmhart, M. Gálová, M. Petronczki, J. Gregan, B. Cetin, I. Mudrak, E. Ogris, K. Mechtler, L. Pelletier, F. Buchholz, K. Shirahige, K. Nasmyth, *Nature* **2006**, *441*, 53.
[52] S. Galander, R. E. Barton, D. A. Kelly, A. L. Marston, *Wellcome Open Res* **2019**, *4*, 29.
[53] M. A. Shonn, R. McCarroll, A. W. Murray, *Genes Dev.* **2002**, *16*, 1659.
[54] B. Lee, B. M. Kiburz, A. Amon, *Curr. Biol.* **2004**, *14*, 2168.
[55] V. L. Katis, J. Matos, S. Mori, K. Shirahige, W. Zachariae, K. Nasmyth, *Curr. Biol.* **2004**, *14*, 2183.
[56] S. Miyazaki, J. Kim, Y. Yamagishi, T. Ishiguro, Y. Okada, Y. Tanno, T. Sakuno, Y. Watanabe, *Genes Cells* **2017**, *22*, 552.
[57] Y.-C. Chen, M. Weinreich, *J. Biol. Chem.* **2010**, *285*, 41244.
[58] T. Sakuno, K. Tada, Y. Watanabe, *Nature* **2009**, *458*, 852.
[59] Y. Xiang, S. Takeo, L. Florens, S. E. Hughes, L.-J. Huo, W. D. Gilliland, S. K. Swanson, K. Teeter, J. W. Schwartz, M. P. Washburn, S. L. Jaspersen, R. S. Hawley, *PLoS Biol.* **2007**, *5*, e323.
[60] A. W. Kerrebrock, D. P. Moore, J. S. Wu, T. L. Orr-Weaver, *Cell* **1995**, *83*, 247.
[61] A. S. Clarke, T. T.-L. Tang, D. L.-Y. Ooi, T. L. Orr-Weaver, *Dev. Cell* **2005**, *8*, 53.
[62] L. S. Goldstein, *Cell* **1981**, *25*, 591.
[63] K. K. Sarangapani, E. Duro, Y. Deng, F. de, L. Alves, Q. Ye, K. N. Opoku, S. Ceto, J. Rappsilber, K. D. Corbett, S. Biggins, A. L. Marston, C. L. Asbury, *Science* **2014**, *346*, 248.
[64] X. Li, R. K. Dawe, *Nat. Cell Biol.* **2009**, *11*, 1103.
[65] T. Chiang, F. E. Duncan, K. Schindler, R. M. Schultz, M. A. Lampson, *Curr. Biol.* **2010**, *20*, 1522.
[66] Y. Watanabe, P. Nurse, *Nature* **1999**, *400*, 461.
[67] Y. Watanabe, S. Yokobayashi, M. Yamamoto, P. Nurse, *Nature* **2001**, *409*, 359.
[68] S. Yokobayashi, M. Yamamoto, Y. Watanabe, *Mol. Cell. Biol.* **2003**, *23*, 3965.
[69] A. Kagami, T. Sakuno, Y. Yamagishi, T. Ishiguro, T. Tsukahara, K. Shirahige, K. Tanaka, Y. Watanabe, *EMBO Rep.* **2011**, *12*, 1189.
[70] S. Ogushi, A. Rattani, J. Godwin, J. Metson, L. Schermelleh, K. Nasmyth, *bioRxiv* **2020**, *106*, 2020.02.06.935171.
[71] A. Tóth, K. P. Rabitsch, M. Gálová, A. Schleiffer, S. B. Buonomo, K. Nasmyth, *Cell* **2000**, *103*, 1155.

[72] F. Monje-Casas, V. R. Prabhu, B. Lee, M. Boselli, A. Amon, *Cell* **2007**, *128*, 477.
[73] K. P. Rabitsch, M. Petronczki, J. P. Javerzat, S. Genier, B. Chwalla, A. Schleiffer, T. U. Tanaka, K. Nasmyth, *Dev. Cell* **2003**, *4*, 535.
[74] M. Petronczki, J. Matos, S. Mori, J. Gregan, A. Bogdanova, M. Schwickart, K. Mechtler, K. Shirahige, W. Zachariae, K. Nasmyth, *Cell* **2006**, *126*, 1049.
[75] K. D. Corbett, C. K. Yip, L.-S. Ee, T. Walz, A. Amon, S. C. Harrison, *Cell* **2010**, *142*, 556.
[76] K. D. Corbett, S. C. Harrison, *Cell Rep.* **2012**, *1*, 583.
[77] S. Sarkar, R. T. Shenoy, J. Z. Dalgaard, L. Newnham, E. Hoffmann, J. B. A. Millar, P. Arumugam, *PLoS Genet.* **2013**, *9*, e1003610.
[78] R. Plowman, N. Singh, E. C. Tromer, A. Payan, E. Duro, C. Spanos, J. Rappsilber, B. Snel, G. J. P. L. Kops, K. D. Corbett, A. L. Marston, *Chromosoma* **2019**, *128*, 331.
[79] R. K. Clyne, V. L. Katis, L. Jessop, K. R. Benjamin, I. Herskowitz, M. Lichten, K. Nasmyth, *Nat. Cell Biol.* **2003**, *5*, 480.
[80] Q. Ye, S. N. Ur, T. Y. Su, K. D. Corbett, *EMBO J.* **2016**, e201694082.
[81] L.-I. Wang, A. Das, K. S. McKim, *PLoS Genet.* **2019**, *15*, e1008072.
[82] S. Klapholz, R. E. Esposito, *Genetics* **1980**, *96*, 567.
[83] O. Argüello-Miranda, I. Zagoriy, V. Mengoli, J. Rojas, K. Jonak, T. Oz, P. Graf, W. Zachariae, *Dev. Cell* **2017**, *40*, 37.
[84] S. Chu, J. DeRisi, M. Eisen, J. Mulholland, D. Botstein, P. O. Brown, I. Herskowitz, *Science* **1998**, *282*, 699.

 

## Appendix

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|---|---|
| *Chiasmata* | the cytological manifestation of reciprocal crossover between DNA molecules from homologous chromosomes. Chiasmata result from homologous recombination and serve to physically link homologs together. |
| *Cohesin* | a ring-shaped protein complex formed by the SMC1, SMC3 and a kleisin subunit (such as Rec8), together with accessory subunits. It is thought to topologically embrace sister chromatids. |
| *DNA replication origins* | sites on the DNA where replication is initiated. |
| *Gametogenesis* | the process by which a diploid mother cell undergoes meiosis and developmental differentiation to form haploid male and female germ cells. |
| *Haploinsufficiency* | a situation in which a heterozygous loss-of-function mutation of a gene is insufficient to provide normal cellular function. |
| *Holliday junction* | a temporary structure formed from two homologous DNA molecules during genetic recombination, which may serve the exchange of genetic information between the two molecules. |
| *Holocentric chromosomes* | chromosomes that lack a distinct centromere and thus have multiple microtubule attachment sites along the length of the chromosome. |
| *Kinetochore* | a proteinaceous structure that assembles on centromeres and serves as attachment site for microtubules during mitotic and meiotic chromosome segregation |
| *Synaptonemal complex* | a proteinaceous structure connecting two homologous chromosomes during meiotic prophase. It serves to facilitate the events of homologous recombination. |